Alkali extraction of archaeological and geological charcoal: Evidence for diagenetic degradation and formation of humic acids

Philippa L. Ascough[*],
SUERC
Scottish Enterprise Technology Park,
Rankine Avenue, East Kilbride G75 0QF, UK

Michael I. Bird
School of Earth and Environmental Sciences
James Cook University PO Box 6811,
Cairns, Queensland, 4870, Australia

S. M. Francis[3], T. Lebl[3]
Department of Chemistry
University of St. Andrews
St. Andrews, Fife KY16 9ST, UK

*corresponding author email: p.ascough@suerc.gla.ac.uk
Present address: SUERC, Scottish Enterprise Technology Park, Rankine Avenue, East Kilbride G75 0QF, UK

Abstract

Charcoal forms a crucial source of archaeological and palaeoenvironmental data, providing a record of cultural activities, past climatic conditions and a means of chronological control via radiocarbon (^{14}C) dating. Key to this is the perceived resistance of charcoal to post-depositional alteration, however recent research has highlighted the possibility for alteration and degradation of charcoal in the environment. An important aspect of such diagenesis is the potential for addition of exogenous "humic acids' (HA), to affect the accuracy of archaeological and palaeoenvironmental reconstructions based upon chemical analyses of HA-containing charcoal. However the release of significant quantities of HA from apparently pristine charcoals raises the question whether some HA could be derived via diagenetic alteration of charcoal itself. Here we address this question through comparison of freshly-produced charcoal with samples from archaeological and geological sites exposed to environmental conditions for millennia using elemental (C/H/O) and isotopic ($\delta^{13}C$) measurements, Fourier Transform Infrared Spectroscopy (FTIR) and proton Liquid-State Nuclear magnetic Resonance (^{1}H-NMR). The results analyses show that the presence of highly carboxylated and aromatic alkali-extractable HA in charcoal from depositional environments can often be attributable to the effects of post-depositional processes, and that these substances can represent the products of post-depositional diagenetic alteration in charcoal.

Keywords

Charcoal, Humic Acid, Oxidative Degradation, Radiocarbon

Introduction

Charcoal is one of the most common materials recovered from archaeological deposits, providing key proxy data for reconstructions of plant resource use and palaeoenvironments, plus chronological information via radiocarbon (^{14}C) dating. Plant-derived charcoal is formed when biomass is exposed to elevated temperatures in conditions of restricted oxygen (pyrolysis). Carbon (C) content increases, while oxygen (O) and hydrogen (H) content decrease as lignocellulosic structures degrade and chemically stable aromatic rings are formed. As pyrolysis temperatures increase these aromatic rings coalesce into polyaromatic configurations, and increasingly form ordered microcrystalline domains, conferring even higher chemical stability (Eckmeier et al., 2007).

Charcoal therefore appears highly resistant to post-depositional alteration (e.g. Czimczik et al., 2005; Preston and Schmidt, 2006), and is often regarded as virtually chemically inert. However this assumption is challenged by evidence suggesting alteration and degradation of charcoal in a range of environmental deposits. This appears to involve oxidative "weathering", with addition of oxygen to the charcoal aromatic skeleton, and an increase in carboxylic (COOH and COO^-) groups (Cohen-Ofri *et al*., 2006; Ascough *et al*., 2010a). Physical degradation is also observed, with extreme net effects upon apparent charcoal abundance, resulting in near complete loss of charcoal from some archaeological deposits and savannah soils (Bird *et al*., 1999; 2002).

A key concern in analysis of charcoal is the potential for post-depositional addition of exogenous material to charcoal samples, as the presence of such material could compromise the accuracy of charcoal chemical analyses. Examples of such analyses include the use of charcoal stable C isotopic composition ($\delta^{13}C$) as a proxy climatic record, if contaminants are present with significantly different $\delta^{13}C$ to that of the charcoal (McCarroll and Loader, 2004, Lichtfouse, 2000). Similarly, addition of exogenous material with a different ^{14}C age to the original charcoal would produce erroneous results (Gillespie, 1997; Alon et al., 2002). A major source of such material in environmental deposits is "humic acids' (HA). These are traditionally defined as alkali-soluble, but acid insoluble products of organic matter humification processes, with formation mediated by soil moisture, pH, temperature, microbial action and inorganic components (Cook, 1964). HA are typically isolated and characterized from

samples by extraction with alkaline solution (typically between 0.1 and 2.0 M NaOH) and acidification of the extract supernatant to pH 1-2. The fraction remaining in the acidified solution is called fulvic acid, and the organic precipitate produced by acidification is called HA (Cook et al., 1998). Analysis of compounds isolated in this way demonstrates that HA are a chemically complex, highly heterogeneous group comprising large macromolecules with substituted oxygenated functional groups, connected by aliphatic and ether linkages (Grasset and Ambles, 1998; Chen et al., 2007).

HA extraction as described above is commonly performed if significant HA contamination is suspected, and is an essential step in pre-treatment of charcoal for ^{14}C measurement (e.g. McGeehin et al., 2001; Miyairi et al., 2004). As a consequence it is sometimes noted that charcoal samples release a large quantity of dark-coloured HA compounds during treatment, often resulting in complete sample break-up and dissolution. This behaviour is not usually predictable from initial sample macrostructure, with significant quantities of HA often released from apparently pristine charcoals (Hedges et al., 1989; Rebollo et al., 2008). This raises the question whether some HA extracted from charcoal could actually have an endogenous origin, and be the result of charcoal diagenesis, rather than represent the introduction of exogenous material. This possibility is supported by analysis of HA extracted from charcoal and fire-affected soils. Substances consistent with HA have been extracted from fresh laboratory-produced charcoal (Trompowsky et al., 2005; Ponomarenko and Anderson, 2001; Kumada, 1983), although this can require the use of strong oxidizing agents (e.g. Haumaier and Zech, 1995). These are similar to HA extracted from soils subjected to high temperatures (e.g. volcanic ash soils) (Kumada 1983; Kramer et al., 2004; Shindo *et al*., 1986). This indicates that some residues of biomass burning either initially conform to the definition of alkali-extractable HA, or become so after periods of deposition in mineral soil (c.f. Kaal *et al*., 2007).

The possibility that HA extracted from charcoal could represent endogenous material has importance for understanding of both the potential products of post-depositional diagenesis of charcoal, and for understanding how such products might behave in the environment. This is of importance as charcoal diagenesis appears mediated to some extent by environmental conditions, in particular pH (e.g. Braadbaart *et al*., 2009). Charcoal-derived HA may therefore display pH-dependant solubility, and hence be mobilized away from the initial depositional site. If such

material also had high aromaticity, it would also be likely to be environmentally recalcitrant and persist for extended periods of time. In addition, the presence of charcoal-derived HA could serve as an indicator for alteration and potential loss of charcoal from a deposit.

Key questions are therefore: (i) whether a difference exists between material defined as HA in freshly-produced charcoal versus that in charcoal exposed to environmental conditions, and (ii) whether evidence exists that HA extracted from charcoal exposed to environmental conditions originates from endogenous sources. We address these questions through comparison of freshly produced charcoal with samples from archaeological and geological sites exposed to environmental conditions for millennia. Analyses are made of the charcoal before and after HA extraction, and of HA precipitated from the alkali extraction supernatant. Elemental (C/H/O) and isotopic ($\delta^{13}C$) measurements together with Fourier Transform Infrared Spectroscopy (FTIR) and proton Liquid-State Nuclear magnetic Resonance (^{1}H-NMR) are used to characterize the chemical form of these materials, and assess the evidence for derivation of the extracted HA from the charcoal. The findings from this investigation have direct implications and potential benefits for analysis of charcoal samples from environmental deposits.

Methodology

Sample material

Two types of sample material were used in this study; fresh laboratory-produced charcoal (Lab_{Char}), and charcoal from archaeological and geological sites (Env_{Char}). Lab_{Char} samples were produced from *Pinus sylvestris* (Scots Pine) and *Rhizophora apiculata* (Mangrove), representing low-density softwood and high-density hardwood, respectively. Briefly, after removal of the bark, charcoal was produced from $1cm^2$ cubes of a homogenized wood sample in a controlled-atmosphere rotary furnace (Carbolite™) for 60 minutes at 300˚C, 400˚C, 500˚C, or 600˚C, resulting in eight Lab_{Char} samples (see Table 1). For further information on experimental production of these materials see Ascough et al., (2008).

Env_{Char} samples varying in age from modern to 50 Ka BP were obtained from a range of depositional environments in ecozones ranging from low latitude tropics (Northeast Brazil) to high latitude sub-Arctic (Iceland) (see Table 1). Radiocarbon (^{14}C) age measurements are available for all samples with the exception of Env-6 and Env-8, where age assessments are made on stratigraphical grounds. An AMS ^{14}C age measurement for Env-9 was obtained in the course of the present study at the Oxford Radiocarbon Accelerator Unit (ORAU), following standard acid-base-acid pretreatment, and is presented in Table 1. Further information on the depositional setting of samples Env-1 to Env-6 is detailed in Ascough et al., (2010a). Env-7 was obtained from ephemeral post-holes and pits at the Royal Hotel site in St Peter Port, Guernsey UK (Sebire, 2002; 2005); Env-8 was obtained from acidic soils developed on granite at the Roman to Mediaeval site of Carvalhais, Portugal (Vieira, 2006), Env-9 was obtained from within deposits of sand and clay in the calcareous rock shelter of Toca Nova do Inharé, northeast Brazil (Guidon, pers. Comm.) All samples selected possessed good structural integrity, clearly preserved plant macrostructure, and did not display any evidence of physical degradation.

Prior to analysis, all charcoal samples were air dried and lightly crushed to pass a 500μm sieve. Soil carbonates and calcitic ash were removed by placing the charcoal in 1M HCl for 24 hours at room temperature, followed by neutralization with Milli-Q™ ultrapure water and oven-drying at 40˚C. After drying to a constant weight charcoal ash content was determined via loss on ignition (see Ascough et al., 2010a). Ash contents were generally <1%, however in Env-7 and Env-9 ash contents > 5%

presumably reflect soil mineral contents not removed during initial pre-treatment. All elemental abundances were subsequently corrected for ash content.

Alkali extraction

Alkali extractions were performed using ~0.1g of prepared charcoal in 40 ml of 1.0 M NaOH solution. This was placed in a capped centrifuge tube in a temperature-controlled incubator shaker at 60˚C/120 rpm for 24 hours. The solid charcoal residue was separated from the extraction supernatant by filtration through Whatman glass microfibre filters. Care was taken to ensure all visible charcoal residue was cleaned and retained from the filter papers, and the charcoal residue was then washed three times with Milli-Q^{TM} ultrapure water, transferred to 7ml pre-cleaned glass vials by pipette and freeze-dried before weighing to calculate mass loss during extraction. HA removed from the charcoal during extraction were reprecipitated via acidification of the extraction supernatant with 36% HCl to achieve a pH of 2, and incubated at 60^0C/120 rpm for 24 hours. HA precipitated by the end of this period was transferred by pipette into visking® dialysis tubing (molecular weight cut-off 10,000 Dalton) and dialysed against deionised water, in order to ensure that HCl was not reprecipitated upon drying. The dialysis solution was tested for salts every 4-6 hours using silver nitrate. When the test was negative for Cl^- ions the precipitates were transferred to 7ml glass vials and freeze-dried.

Elemental analysis and stable isotopic composition

In freshly produced charcoal the atomic H/C and O/C ratios are closely correlated with production temperature (Ascough et al., 2010a), where H/C ratio provides a measure of pyrolysis efficiency (Nguyen et al., 2004). In charcoal exposed to natural environmental conditions O/C ratios appear to also reflect the degree of sample oxidation, where higher O/C ratios indicate oxidative alteration (Cohen-Ofri et al., 2006; Ascough et al., 2010a). The atomic H/C and O/C ratios of untreated charcoal and charcoal following extraction were compared to examine whether these values were correlated with the amount and nature of HA released from samples during treatment. The carbon isotopic value ($\delta^{13}C$) of individual plants can vary by several ‰ even when grown in close proximity, and processes of biomass decomposition and humification substantially alter plant isotopic composition (Wedin et al., 1995; McCarroll and Loader, 2004;). Therefore, if significant $\delta^{13}C$ differences existed

between the untreated charcoal, charcoal residue and HA extracted from the charcoal, this would be suggestive of an exogenous origin for the HA.

Elemental (C, O, H) abundances (wt %) and isotopic ($\delta^{13}C$) values were obtained for unextracted charcoal and for extracted charcoal residue. Due to low mass of material precipitated from the extraction supernatant, only C elemental and isotopic analysis was possible on these samples. Atomic H/C and O/C ratios of unextracted samples of Lab_{Char}, Env-1, Env-2, Env-4, Env-5 and Env-6 are reported in Ascough et al., (2010a). For all other samples, C content (wt %) was measured using a Costech elemental analyser (EA), while O and H content (wt %) was measured using a ThermoFinnigan High-Temperature Conversion Elemental Analyzer (TC/EA), with a zero-blank auto-sampler installed on both the EA and TC/EA. Elemental abundances were calculated by comparing the gas pulse peak area to acetanilide (%C: 71.09%, %O: 11.84%, %H: 6.71), with an external reproducibility of better than 0.5% for C, 0.7% for O, and 0.4% for H. The EA was coupled via a ConFloII to a ThermoFinnigan Delta^{plus} XL on which $\delta^{13}C$ was measured using Continuous-Flow Isotope Ratio Mass Spectrometry (CF-IRMS). Values of $\delta^{13}C$ were obtained on duplicate samples that were measured with a mixture of laboratory standards and blanks. Sample $\delta^{13}C$ was calculated using three standards: acetanilide ($\delta^{13}C$: -30.11‰,), a commercially available protein (B2155: $\delta^{13}C$: -26.98‰) and a C_4 cane sugar/uric acid mix (Tesco: $\delta^{13}C$: -12.02‰). Precisions (SD) on internal standards were better than ±0.2‰ (1σ) for $\delta^{13}C$. The $\delta^{13}C$ values for the samples are reported as per mil (‰) deviations from the VPDB international standard.

Fourier transform infrared spectroscopy (FTIR)

The mid-infrared region of the electromagnetic spectrum (~4000–400 cm^{-1} (equivalent to 30–2.5 μm)) is used to study the structure of chemical compounds in biomass via Fourier Transform Infrared Spectroscopy (FTIR) (e.g. Moore and Owen, 2001). This technique has been successfully used to characterize the chemical composition of charcoal samples, including material from environmental deposits (Nishimiya et al., 1998; Guo and Bustin, 1998; Cohen-Ofri et al., 2006). FTIR is also used in characterization of HA extracted from soils and sediments (e.g. Schmitt-Kopplin et al., 1998; González Pérez et al., 2004). FTIR spectra of Lab_{Char} and Env_{Char} were compared before and after alkali treatment to determine whether initial chemical

differences between these sample types were correlated with HA release during treatment. The FTIR spectra of extracted HA were examined to determine whether material extracted from different charcoals was consistent, and whether any chemical forms that could be attributed to exogenous material could be identified.

Freeze-dried samples were diluted by grinding with KBr powder (Sigma-Aldrich Inc., 99+% FT-IR grade purity), and pressed into pellets prior to analysis. Spectra were recorded on a Nicolet FTIR instrument at the University of St. Andrews. Absorbance values were determined between 4000 and 400 cm^{-1}. Data processing was performed using OMNIC software with identification of spectral bands by comparison with published assignments.

^{1}H-Solution state Nuclear Magnetic Resonance (^{1}H-NMR)

Proton Nuclear Magnetic Resonance (^{1}H-NMR) spectra of most organic compounds are characterized by chemical shifts in the range +12 to -4 ppm, and this technique has been successfully used in the characterization of HA from environmental deposits (e.g. Kang et al., 2002). ^{1}H-NMR spectra of obtained of HA precipitated from extraction supernatant of charcoal samples to assess whether the chemical structure of these materials was indicative of an exogenous origin. Sample material was characterized after dissolution in $CDCl_3$, and ^{1}H NMR spectra were recorded in a 5-mm tunable probe with a Bruker AVANCE 300 MHz spectrometer. The resulting chemical shifts are given in ppm with respect to Me_4Si.

Results

Mass change, elemental and isotopic analyses

Laboratory charcoals (Lab_{Char})

Mangrove Lab_{Char} formed at ≤400˚C (i.e. M-400, M-500, M-600) lost ≤4% of starting mass during NaOH extraction, and pine Lab_{Char} formed at ≤400˚C (i.e. P-400, P-500, P-600) lost ≤7% of starting mass. Mass loss was higher in 300˚C Lab_{Char} samples, with losses of 8% in M-300 and 32% in P-300. Very minor quantities of material, (sufficient only for ^{1}H-NMR analysis), were precipitated from the NaOH extraction supernatant of 300˚C Lab_{Char} samples (P-300 and M-300), in the form of a fine white powder.

Overall oxidation and dehydrogenation of Lab_{Char} was observed following NaOH treatment, with an increase in O/C in all samples, and a decrease in H/C in all samples (See Table 2, Figure 1). There is a strong relationship between O/C and H/C ratio in the Lab_{Char} samples (Ascough et al., 2010a), which remains after NaOH extraction, as demonstrated by the regression lines for these sample groups shown on Figure 1. The changes in O/C or H/C following NaOH extraction are slightly lower in samples prepared at higher temperatures, however there is no clear overall relationship with either production temperature or species. Following NaOH extraction Lab_{Char} $\delta^{13}C$ is not significantly different from the unextracted charcoal except in P-500, where an increase of +0.54‰ is observed (Table 2).

Environmental charcoals (Env_{Char})

Sample Env-1 lost only 2% of starting mass during NaOH extraction (Table 2) and no material was precipitated from the extraction supernatant. All other Env_{Char} samples exhibited much higher mass losses of 14-52% during alkali extraction (Table 2) and significant quantities of brown-coloured HA were precipitated, which took the form of a fine powder once freeze-dried. NaOH extraction of Env_{Char} also resulted in overall oxidation and dehydrogenation of the samples, as observed in the change in O/C and H/C ratios (Figure 1, Table 2). Env_{Char} mass losses during NaOH treatment are not strongly influenced by O/C ratio of the initial charcoal, but are positively

correlated with H/C ratio. The change in O/C ratio within the Env_{Char} group is similar to that observed in the Lab_{Char}, although the Env_{Char} group shows slightly smaller changes in H/C ratio following alkali treatment than is observed in the Lab_{Char} group. Following extraction, the majority of Env_{Char} $\delta^{13}C$ values were not significantly different to unextracted samples, although the $\delta^{13}C$ of Env-6 and Env-8 increased by +0.49‰ and +0.70‰, respectively (Table 2).

The HA precipitate from Env_{Char} samples was sufficient for %C and $\delta^{13}C$ measurement in all samples except Env-4. Carbon abundance in the HA ranged from 42% to 58% (Table 3). $\delta^{13}C$ values were not significantly different to that of the unextracted material and extraction residues, with the exception of Env-8, where the $\delta^{13}C$ of the HA was higher than that of the un-extracted charcoal by +0.63‰ (Table 3).

FTIR

Peak assignments are detailed in Table 4. Signals in the 2000-4000 cm^{-1} region include a broad absorbance centred at 3460 cm^{-1} for O–H stretching, possibly including H_2O, alcoholic OH, phenolic OH, and/or carboxylic OH (Marshall et al., 2005). Further signal relates to alkyl group absorbance at 2925 cm^{-1} (antisymmetric CH_2 stretch) 2855 cm^{-1} (symmetric CH_2 stretch) (Marshall et al., 2005). However the majority of information was contained in the 'fingerprint' region between 600-2000 cm^{-1}; this information is discussed in the analysis of the results below and presented in Figures 2, 3, 4 and 5.

Laboratory charcoals (Lab_{Char})

The spectra of the 600°C Lab_{Char} samples (Figure 2) are dominated by signal at 1570 cm^{-1} for aromatic C=C ring structures, peaks at 700-900 cm^{-1} for aromatic CH deformation (Guo and Bustin, 1998). Spectra also contain a broad, indistinct signal centred on 1190 cm^{-1}, which has been assigned to the C-O stretch of esters, phenols and ethers (Marshall *et al*., 2005). In contrast, the 300°C Lab_{Char} samples consist of an alkyl aromatic structure containing oxygenated functional groups. These include lignocellulosic and aliphatic material, with peaks for cellulose CH_2 symmetric bending (1420-1430 cm^{-1}) plus C-O-C and alcohol OH groups at 1030-1160 cm^{-1} (Guo and Bustin, 1998; Colom and Carrillo, 2002). Lignin aromatic stretching is

visible at 1510 cm^{-1}, and aliphatic CH_x deformation at 1450 cm^{-1} (Owen and Thomas, 1989). The 300°C Lab_{Char} samples contain bands at 1600 cm^{-1} and at 1380 cm^{-1}; the presence of both of these in one spectra are attributable to ionized carboxyl (COO^-) groups (Benites et al., 2005; Rebollo *et al*., 2008), although the 1600 cm^{-1} band also contains signal from aromatic skeletal C=C (Nishimiya *et al*., 1998). Likewise, the presence of bands at 1700 cm^{-1} and 1250-1270 cm^{-1} demonstrates a significant content of carboxylic acids (COOH). The 300°C Lab_{Char} samples also contain aromatic C-O (1200-1220 cm^{-1}) and aromatic CH (700-900 cm^{-1}) (Guo and Bustin, 1998; Nishimiya *et al*., 1998; Stevenson, 1994).

NaOH treatment produces little change in the 600°C pine (P-600) Lab_{Char} spectra, although there is some evidence of an increase in aromatic C-O, with development of a minor peak at 1200-1220 cm^{-1}, and increased importance of the C-O stretch centred on 1190 cm^{-1}. In the mangrove 600°C Lab_{Char} spectra (M-600), these changes are also observed, but appear more pronounced. After NaOH treatment, the 300°C Lab_{Char} spectra show significant alteration (Figure 2). The COOH peaks are removed, due to the neutralizing action of Na^+ cations from the alkali (Rebollo et al., 2008). P-300 retains strong COO^- signal (1600 cm^{-1} and 1380 cm^{-1}), together with remaining signal for cellulose (1030-1160 cm^{-1}), lignin (1510 cm^{-1}) and aliphatic CH_x deformation. In contrast, after NaOH treatment 300°C mangrove Lab_{Char} (M-300) resembles the 600°C Lab_{Char} spectra, retaining only a peak at 1560 cm^{-1}, a broad signal centred on 1190 cm^{-1} and peaks for aromatic CH at 700-900 cm^{-1}. In this sample all distinct aliphatic and lignocellulosic peaks are removed.

Environmental charcoals (Env_{Char})

Env-1 closely resembles the 600°C Lab_{Char} samples, with a highly aromatic structure containing aromatic CH groups. However, all other Env_{Char} samples consist of a highly carboxylated aromatic structure (Figure 3). These spectra contain strong signal for aromatic C-O (1220 cm^{-1}) and aromatic CH (700-900 cm^{-1}), but are dominated by signal for COOH and COO^- groups (1700 cm^{-1}, 1600 cm^{-1}, 1380 cm^{-1} and 1250-1270 cm^{-1}). Only samples Env-5 and Env-7 contain signal for oxygenated cellulose functional groups, with this signal being much stronger in Env-5. Samples Env-2 and Env-5 contain signal for cellulose CH_2 bending (1420-1430 cm^{-1}) and lignin (1510 cm^{-1}). In addition, Env-2 also appears to contain C=O of amide groups (1650 cm^{-1}) (Benites et al., 2005).

Following NaOH extraction of Env_{Char} samples the COOH (1700 cm^{-1}) peak is removed and spectra are dominated by COO^- (~1600 cm^{-1} and 1380 cm^{-1}). The centre of the 1600 cm^{-1} peak is generally shifted to lower frequency (i.e. 1560-1580 cm^{-1}), and signal for aromatic C-O is no longer apparent (Figure 4). In samples Env-2, Env-5 and Env-7 lignocellulosic peaks are removed, and signal only remains at ~1190 cm^{-1}, similar to that observed in Lab_{Char} samples. Distinct peaks are visible in all spectra for aromatic CH groups (900−700 cm^{-1}) (Guo and Bustin, 1998; Várhegyi *et al*., 1998).

HA precipitated from Env_{Char} following NaOH extraction supernatant consists of a highly carboxylated aromatic structure, dominated by COOH and COO^- peaks (Figure 5). There is a clear contribution from aromatic CH (700-900 cm^{-1}), particularly at 770 cm^{-1} (aromatic rings with 3-4 adjacent H atoms, Guo and Bustin, 1998). Aliphatic and lignocellulosic material is only apparent in trace amounts in the extract from Env-5, which contains signal for lignin (1510 cm^{-1}), aliphatic CH_x (1450 cm^{-1}), and cellulose glycosidic links (1117 cm^{-1} and 1030 cm^{-1}). All Env_{Char} HA precipitates are very similar with the exception of Env-8, where the peak for COOH at ~1700 cm^{-1} is missing.

Solution NMR

The large peak in the spectra at 4.73 is derived from H_2O, and the peak at 3.108 relates to an unidentified compound that is consistent between all spectra and appears to represent a feature of the solvent. Otherwise the overall charcoal HA ^{1}H-NMR spectra contain a broad range of poorly-resolved peaks. This is insufficient information to assign specific chemical compounds, however it is possible to divide the signal into three main groups of chemical environments: aliphatic protons (1.5-2.5 ppm), protons associated with carbohydrates (3.0-4.5 ppm) and aromatic protons (6.0-8.5 ppm). This is sufficient to distinguish between the signal from HA isolated from (300ºC) Lab_{Char} and that isolated from the Anc_{hor}, as the relative proportion of spectra signal attributable to the three chemical environments varies between samples (Figure 6, Table 5). The majority of signal from the 300ºC Lab_{Char} HA is concentrated in the carbohydrate region; these spectra do not contain any significant aromatic signal, indicating this material is comprised of carbohydrate fragments (Figure 6). In contrast the Env_{Char} samples contain varying proportions of aromatic components, along with some carbohydrate and aliphatic signal. Integration of the different regions for such spectra is approached with caution, as the broadness of the signal introduces higher

levels of uncertainty to the figures for integration. However, even such a broad integration highlights the differences between HA extracted from Lab_{Char} and Anc_{hor} samples (see Table 5).

Interpretation

Lab_{Char} samples

During charcoal formation the majority of plant lignocellulosic material decomposes at <400°C, after which the estimated size of polyaromatic domains and degree of chemical structural order increases dramatically (Solum et al., 1995). In fresh charcoal produced at ≥400°C it appears that very little material is NaOH-extractable, and only minor chemical changes occur during extraction. Oxidation is likely to involve insertion of oxygen between organized aromatic layers and at the edges of aromatic groups, while the reduction in H/C ratio indicates demethylation via the loss of any remaining aliphatic material and of substituted groups from the edges of aromatic domains. Demethylation may contribute to the observed minor weight losses in the ≥400°C Lab_{Char}, however it must also be considered that, although care was taken to recover all charcoal residue, some microscopic fraction may have been trapped within the filter papers, contributing to the overall weight losses.

In fresh charcoal produced at 300°C it is clear that thermal decomposition of lignocellulosic fragments is incomplete, and the content of non-aromatic material is much higher. The action of NaOH on lignocellulosic material cleaves ester bonds, removes acetyl groups, solubilizes phenolic material, and ruptures inter-molecular H-bonds (Chesson, 1980). Thermal treatment increases the solubility of cellulose in alkaline solution, and removal of this material explains the larger 300°C Lab_{Char} weight losses (Cao and Tan, 2002). It is suggested that alkali extraction of lignocellulosic biomass produces molecules denoted as HA via polymerisation of small lignin fragments (Zumann and Rupp, 2006). Such materials would contain a significant aromatic component, however the 300°C Lab_{Char} precipitate appears to consist of degraded cellulosic material. This is inconsistent with the physical and chemical appearance of HA extracted from charcoal exposed to environmental conditions.

Chemical changes during alkali extraction do not result in major alteration of the Lab_{Char} $\delta^{13}C$, with a significant isotopic variation only observed in one Lab_{Char} residue (P-500) of +0.54‰. Therefore it does not appear that NaOH treatment of fresh charcoal results in large fractionation effects. The results discussed above suggest in aggregate, that the presence of NaOH-extractable HA in charcoal from depositional environments is most likely attributable to post-depositional processes, and that

variation in $\delta^{13}C$ in charcoal exposed to environmental conditions following NaOH treatment in excess of 0.6‰ appears more likely to relate to either fractionation during diagenesis, or the presence of exogenous compounds.

Env_{Char} samples

Sample Env-1 undergoes little mass loss or chemical change during NaOH treatment, with no release of material identified as HA. This sample is highly aromatic, with O/C and H/C ratios similar to Lab_{Char} samples produced at 600°C; previous reflectance-based production temperature for this sample is calculated at >500°C (Ascough et al., 2010a). The remainder of the Env_{Char} samples (Env-2 to Env-9) possess H/C ratios consistent with formation at lower (300-450°C) temperatures, but higher O/C ratios than predicted from Lab_{Char} samples at these temperatures (Ascough et al., 2010a and present data). This, along with the high degree of carboxylation in these samples, is indicative of oxidative diagenetic alteration (see Cohen-Ofri et al, 2006; Ascough et al., 2010a).

Chemical changes in the Env_{Char} as a result of NaOH treatment appear small, and there are only small differences in changes in atomic ratio during NaOH treatment between the Lab_{Char} and Env_{Char} sample groups. This argues against the removal of significant amounts of exogenous (i.e. non-charcoal) material from the Env_{Char}. Where chemical changes are observed in the extracted residue, these relate predominantly the removal of lignocellulosic material from Env-2, Env-5 and Env-7. This material could originate from the depositional environment (e.g. soil humus), however the 300°C Lab_{Char} analysis demonstrates incomplete thermal degradation of lignocellulosic fragments in charcoal produced at lower temperatures. As reflectance-based production temperatures are 320 ± 29°C and 361 ± 25°C for Env-2 and Env-5 respectively (Ascough et al., 2010a), it is possible that the lignocellulosic component in Env-2, Env-5 and Env-7 is endogenous. This is supported by the observation that removal of these components does not result in a significant change in sample $\delta^{13}C$.

The extracted HA in all cases comprises a highly carboxylated aromatic structure, with low aliphatic content. These substances differ markedly from the carbohydrate fragments isolated from the 300°C Lab_{Char}, (e.g. Figure 6), meaning that a post depositional origin is likely. However, despite the removal of significant quantities (up to 50%) of sample mass, the $\delta^{13}C$ of Env_{Char} following extraction, and

HA only differs in Env-6 and Env-8. The majority of the HA extracted from the Env_{Char} cannot therefore be ascribed to an exogenous source on the basis of isotopic evidence. The HA extracted from the Env_{Char} also differs from that commonly isolated from soils and non-carbonized organic material. This usually contains a prevalence of protein-derived (generally amide) forms of N and some heterocyclic N compounds (e.g. Trompowsky et al., 2005). However, all FTIR peaks in the Env_{Char} HA are related to polycyclic aromatic structures or carboxylic groups, and contain no obvious signal for any forms of N. These features, further indicate that the Env_{Char} HA are more likely to represent the degraded remains of an original charcoal, rather than an influx of exogenous substances into the charcoal from the depositional environment.

Implications for understanding of charcoal diagenesis

The potential for diagenetic alteration of charcoal to produce alkali-extractable HA has important implications in a number of fields. HA extracted from charcoal are often viewed as representing exogenous material, where the release of large quantities of HA is taken as indicative of highly 'contaminated' samples. The results of this study provide support for the interpretation that in at least some samples, the dominant proportion of the material released may originate from the original charcoal. For example, radiocarbon (^{14}C) age measurement of the labile carbon (i.e. non-aromatic) sample component of Env-2 showed no ^{14}C age difference from that of the aromatic char structure (Ascough et al., 2010b). The present study demonstrates that release of large quantities of material during charcoal alkali extraction can provide a useful measure of diagenetic alteration, but importantly that this process should not necessarily be taken as a demonstration of the presence of large quantities of exogenous material without further supporting evidence.

The production of alkali-extractable material in charcoal could clearly result in the loss of charcoal material from a deposit. The mechanism by which this process occurs is unclear, however oxidation reactions appear to be a key factor. This is highlighted by the fact that the Env_{Char} samples which release quantities of HA during NaOH extraction also have high O/C ratios relative to those of freshly produced charcoal over a wide temperature range (300-600°C). Such oxidative degradation seems also to affect the organized phase of some charcoals, resulting in products that resemble HA (Cohen-Ofri *et al*., 2006). Previous work (Ascough et al., 2010a)

suggests that lower temperature charcoal is more susceptible to oxidative degradation, meaning that care should be taken in such deposits where for example, where quantitative measurements of charcoal abundance were being made.

The samples used in this study cover a wide age range; however there does not appear to be a clear correlation between sample age (and hence length of time exposed to environmental conditions), and the proportion of material released during NaOH extraction. It seems more likely that the charcoal production conditions (especially temperature) and the depositional environment, play a stronger role in modulating the processes of charcoal diagenesis, particularly pH and moisture status. For example the presence of water vapour significantly accelerates surface oxidation of carbonaceous materials (Boehm, 1994; Billinge et al., 1984; Adams et al., 1988). Depositional conditions are also likely to affect the dispersion of charcoal degradation products. The presence of different carboxylic units is dependant upon pH, and influences the hydrophilicity of materials (Cozzalino et al., 2001). Hydrophyllic groups aid dispersion in water, for example oxidized carbon blacks form spontaneous colloidal dispersions in water (Donnet 1982). Increased solubility in such materials can also be achieved by oxygen functionalities, such as are inserted during the oxidation process. The hydroxylic, ketonic and carboxylic moieties formed may mean that the HA from charcoal is soluble in a pH-dependant manner. In addition, the carboxylated aromatic chemical structure of these substances are likely to be environmentally recalcitrant (Brodowski et al., 2005), meaning that once oxidized, the degraded charcoal-derived HA is likely to be chemically stable and demonstrate high longevity. Therefore this material, as well as being mobile, may be highly persistent in the environment. A further implication of the research presented here that charcoal-derived HA may itself be subject to extensive remobilization and redeposition within many sedimentary systems.

Conclusions

The results of this study highlight important aspects regarding the impact of exposure to environmental conditions upon the chemical characteristics of charcoal samples. Freshly produced charcoal exposed to temperatures ≥400°C contains little NaOH-extractable material, and while fresh charcoal produced at temperatures <400°C contains variable amounts of NaOH-extractable material, this is dominantly composed of degraded cellulosic fragments. This contrasts sharply with the highly carboxylated aromatic structure of HA extracted from charcoal following exposure to environmental deposits. However, despite the presence of significant quantities of HA in many of these samples, chemical analyses suggest that these substances are frequently derived from diagenetic alteration and degradation of the initial charcoal structure. For example, the carbon stable isotopic composition of HA extracted from charcoal samples from a range of archaeological and geological deposits is dominantly indistinguishable from that of the isolated charcoal residue. Although the precise processes of such alteration are not yet well understood, it is apparent from the results presented here that oxidation reactions, particularly carboxylation, play an important role. These findings are relevant for both archaeological and palaeoenvironmental studies, not least due to the current understanding that release of HA from charcoal samples during alkali treatment represents dominantly exogenous contaminating material. The results of this study suggest that, although the release of HA indicates diagenetic alteration may have affected a charcoal sample, this does not necessarily involve the addition of exogenous material in every case. Instead, it appears that HA extracted from at least some charcoal represents the altered remains of initial plant material. An important consideration is therefore the potential for mobilization and loss of charcoal structures from a deposit. This would affect interpretations based upon measurements of charcoal abundance, but also raises the possibility that charcoal degradation products could be subject to remobilization and redeposition on wide spatial and temporal scales. A key direction for future research in this field appears to be quantification of the effect of specific depositional conditions, including pH and moisture status, upon chemical processes that clearly have a dynamic effect upon charcoal within environmental systems.

Acknowledgements

Funding for this research was provided by NERC standard grant NE/F017456/1 and NERC grant NE/C004531/1. The authors gratefully acknowledge Kim Wilson, University of Glasgow for assistance with elemental analyses, and the excavation directors of archaeological sites mentioned in the text (Brent Alloway, Mike Church, Neide Guidon, Heather Sebire and Marina Vieira). This research represents a contribution from SAGES (The Scottish Alliance for Geosciences, Environment and Society).

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Figure captions

Figure 1: Van Krevelan diagram of Lab_{Char} and Env_{Char} showing atomic O/C and H/C ratios of charcoal prior to and following NaOH extraction. Linear regressions for Lab_{Char} samples prior to and following NaOH extraction are shown. Note the shift in both sample sets to higher O/C and lower H/C ratios following NaOH extraction, indicating oxidation and dehydrogenation. Note also the Env_{Char} sample group shows higher initial oxygen content than the Lab_{Char} group.

Figure 2: FTIR spectra of Lab_{Char} showing charcoal produced at low (300°C) and high (600°C) temperature (A) before alkali (NaOH) extraction and (B) after alkali (NaOH) extraction.

Figure 3: FTIR spectra of Env_{Char} charcoal samples prior to NaOH extraction. Sample labels are given on the figure.

Figure 4: FTIR spectra of Env_{Char} charcoal residue following NaOH extraction. Sample labels are given on the figure.

Figure 5: FTIR spectra of HA material extracted from Env_{Char} charcoal samples. Sample labels are given on the figure.

Figure 6: Examples to enable comparison of ^{1}H-NMR spectra of alkali-extractable material from Lab_{Char} (P-300 and M-300), and Env_{Char} (Env-2 and Env-9).

Tables

Analogue charcoal samples (Lab_{Char})			Environmental charcoal samples (Env_{char})		
Sample code	**Species**	**Temp (°C)**	**Sample code**	**Location**	**Age**
P-300	*Pinus sylvestris*	300	**Env-1**	Maninjau, Sumatra	53400 ± 1400 ^{14}C yrs BP[1]
P-400	*Pinus sylvestris*	400	**Env-2**	Faial island, Azores	1049 ± 24 ^{14}C yrs BP[2]
P-500	*Pinus sylvestris*	500	**Env-4**	Höskulsstaðir, Iceland	895 ± 35 BP[3]
P-600	*Pinus sylvestris*	600	**Env-5**	Toca da Bastiana, Brazil	129.7 ± 0.4 pMC[2]
M-300	*Rhizophora apiculata*	300	**Env-6**	Oursi-hubeero, Burkina Faso	c. 1050 AD[4,5]
M-400	*Rhizophora apiculata*	400	**Env-7**	Royal hotel site, St. Peter Port, Guernsey, UK	6308 ± 36 ^{14}C years BP[6]
M-500	*Rhizophora apiculata*	500	**Env-8**	Carvalhais, Portugal	Undated. Roman-period deposits[7]
M-600	*Rhizophora apiculata*	600	**Env-9**	Toca Nova do Inhare, Brazil	7355 ± 40 ^{14}C years BP (OxA-16027) [8]

Table 1: Descriptions of Lab_{Char} and Env_{Char} charcoal samples used in this study.
[1](Alloway et al., 2004), [2](Ascough et al., 2010b), [3](Church et al., 2007), [4,](Hallier and Petit, 2000); [5](Hallier and Petit 2001), [6](Sebire, 2005), [7](Vieira, 2006), [8] Age obtained at the Oxford Radiocarbon Accelerator Unit (ORAU), this study.

	Charcoal							Alkali-extracted charcoal						
Sample	**% Ash**	**$\delta^{13}C$**	**%C**	**%O**	**%H**	**O/C**	**H/C**	**Weight loss**	**$\delta^{13}C$**	**%C**	**%O**	**%H**	**O/C**	**H/C**
P-300	0	-26.70	59	31	5	0.39	1.05	32	-26.98	58	30	5	0.39	1.00
P-400	0	-27.71	73	18	3	0.19	0.57	1	-27.65	68	27	3	0.30	0.49
P-500	0	-27.76	81	11	3	0.10	0.49	7	-27.22	75	15	3	0.15	0.41
P-600	0	-27.83	85	7	3	0.06	0.37	6	-27.63	87	12	2	0.10	0.24
M-300	0	-27.53	68	22	5	0.25	0.79	8	-27.62	65	27	4	0.31	0.78
M-400	0	-28.31	76	18	3	0.18	0.53	0	-28.59	71	21	3	0.22	0.52
M-500	0	-28.72	80	10	3	0.10	0.50	-1	-28.58	76	14	3	0.14	0.41
M-600	0	-28.50	83	7	2	0.06	0.31	3	-28.33	87	9	2	0.08	0.27
Env-1	0.3	-24.12	82	7	2	0.06	0.33	2	-24.49	77	12	3	0.12	0.41
Env-2	3.4	-22.41	61	32	4	0.39	0.73	52	-22.55	58	31	3	0.40	0.69
Env-4	0.3	-28.76	62	27	3	0.33	0.54	15	-28.29	59	28	3	0.36	0.52
Env-5	0.1	-26.73	58	27	4	0.35	0.75	28	-26.88	58	29	3	0.37	0.68
Env-6	1.4	-24.39	69	20	2	0.22	0.35	18	-24.41	70	25	2	0.26	0.35
Env-7	5.1	-26.90	63	33	3	0.39	0.47	26	-26.85	61	32	3	0.39	0.48
Env-8	3.8	-25.91	64	32	3	0.38	0.54	16	-25.20	61	34	3	0.41	0.50
Env-9	6.4	-27.79	64	27	3	0.32	0.49	14	-27.83	63	31	3	0.37	0.48

Table 2: Isotopic values and elemental composition of Lab_{Char} and Env_{Char} prior to and following alkali extraction by NaOH.

Sample	%C	%O	O/C	$\delta^{13}C$
Env-1	*	*	*	*
Env-2	58	27	0.35	-22.45
Env-4	*	*	*	*
Env-5	58	54	0.70	-26.62
Env-6	56	*	*	-24.16
Env-7	55	33	0.44	-26.78
Env-8	42	*	*	-25.28
Env-9	56	33	0.45	-27.69

Table 3: Isotopic and elemental values for material extracted from Env_{Char} samples by NaOH.

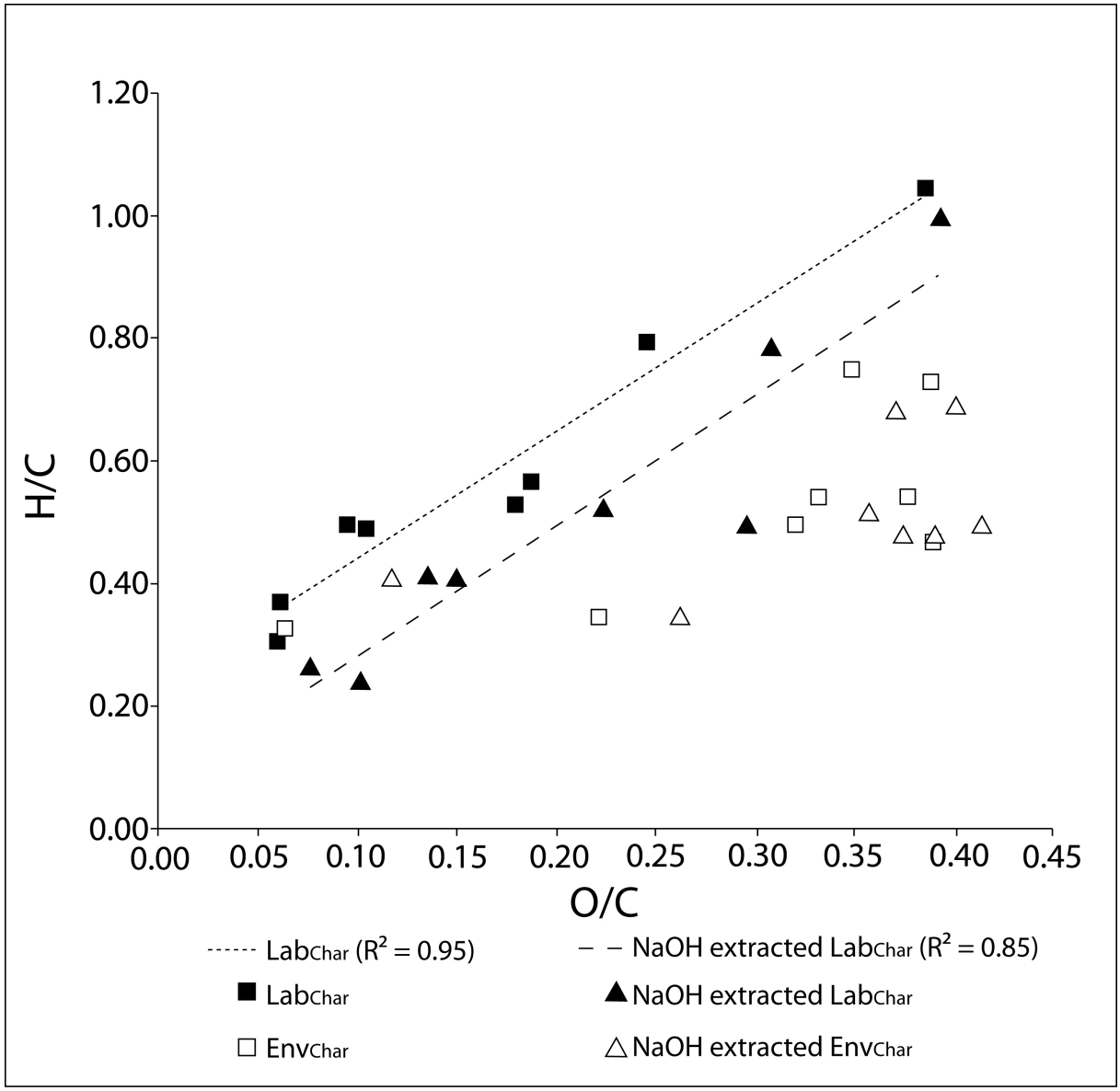
*Insufficient material for analysis.

Bands (cm^{-1})	**Assignment**
3400–3320	–OH stretching
3000–2800	aliphatic CHx stretching vibration
1700	Aromatic carbonyl/ carboxyl (COOH) C=O stretching
1650	C=O of amide groups
1610	COO^-
1600-1570	Aromatic C=C ring stretching
1510	Aromatic C=C ring stretching (lignin)
1450	aliphatic CH_x deformation
1420-1430	cellulose CH_2 symmetric bending
1380	COO^-
1270-1250	O–H deformation in COOH
1200-1220	aromatic C-O
1190	C-O stretch of esters, phenols and ethers
1030-1160	Aliphatic ether C–O– and alcohol C–O stretching
800-900	aromatic CH deformation
870	1 adjacent H deformation
810	2 adjacent H deformation
750	3 – 4 adjacent H deformation

Table 4 : IR assignments. After Guo and Bustin, 1998; Bustin and Guo, 1999; Trompowsky et al., 2005; Benites et al., 2005).

Sample	Aromatic (6.0-8.5 ppm)	Carbohydrate (3.0-4.5 ppm)	Aliphatic (1.5-2.5 ppm)
PC-300	0	98.78	1.62
MC-300	0	89.89	10.11
Env-2	32.41	37.75	29.83
Env-4	33.41	66.59	0
Env-5	19.85	50.66	29.49
Env-6	35.09	60.70	4.21
Env-7	18.27	76.59	5.14
Env-8	33.36	53.25	13.39
Env-9	70.82	24.53	4.65

Table 5: Integration of ^{1}H-NMR spectral regions for different chemical compound classes within HA extracted from Env_{Char} samples.



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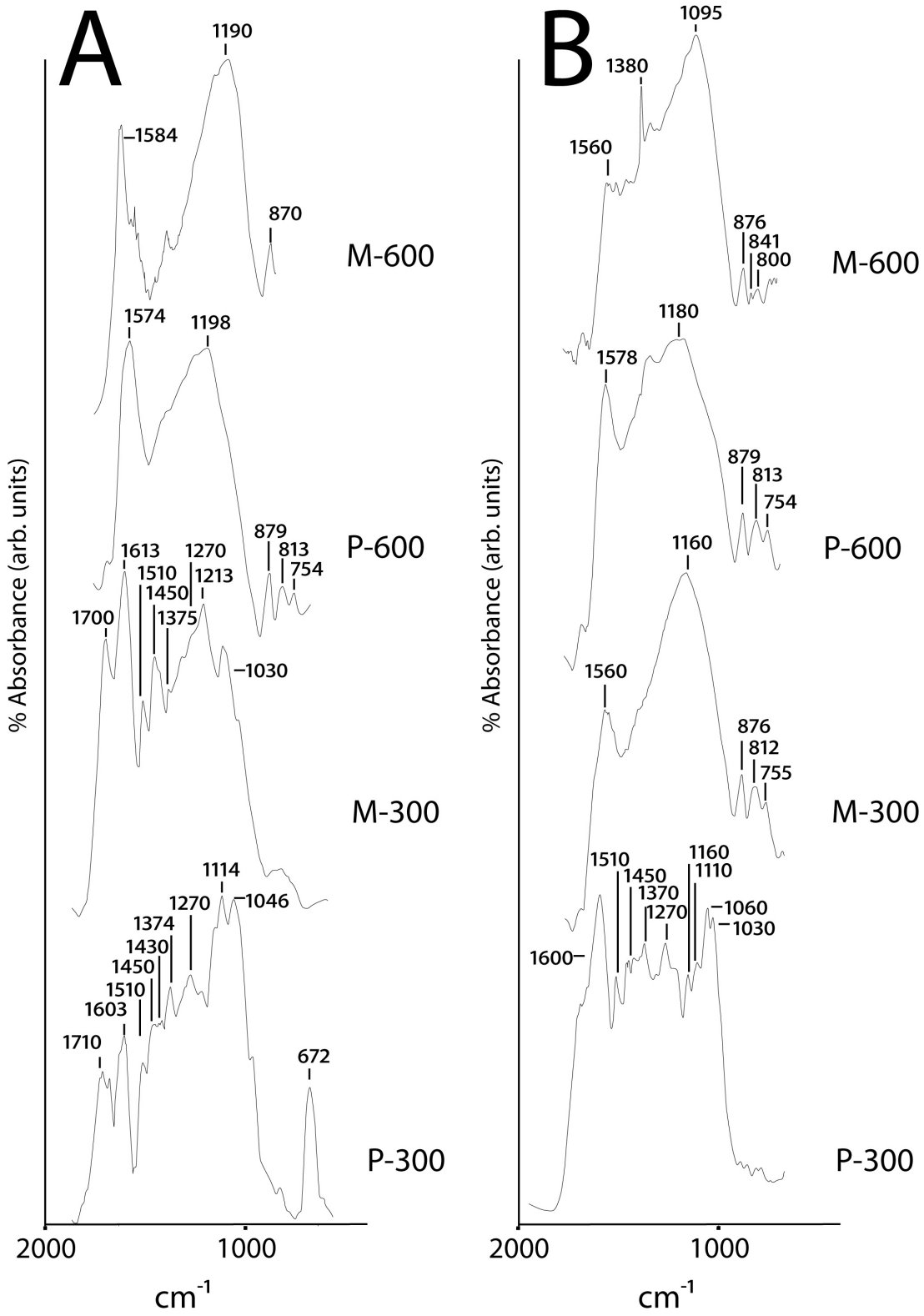


Figure 2: FTIR spectra of Lab_{Char} showing charcoal produced at low (300°C) and high (600°C) temperature (A) before alkali (NaOH) extraction and (B) after alkali (NaOH) extraction.

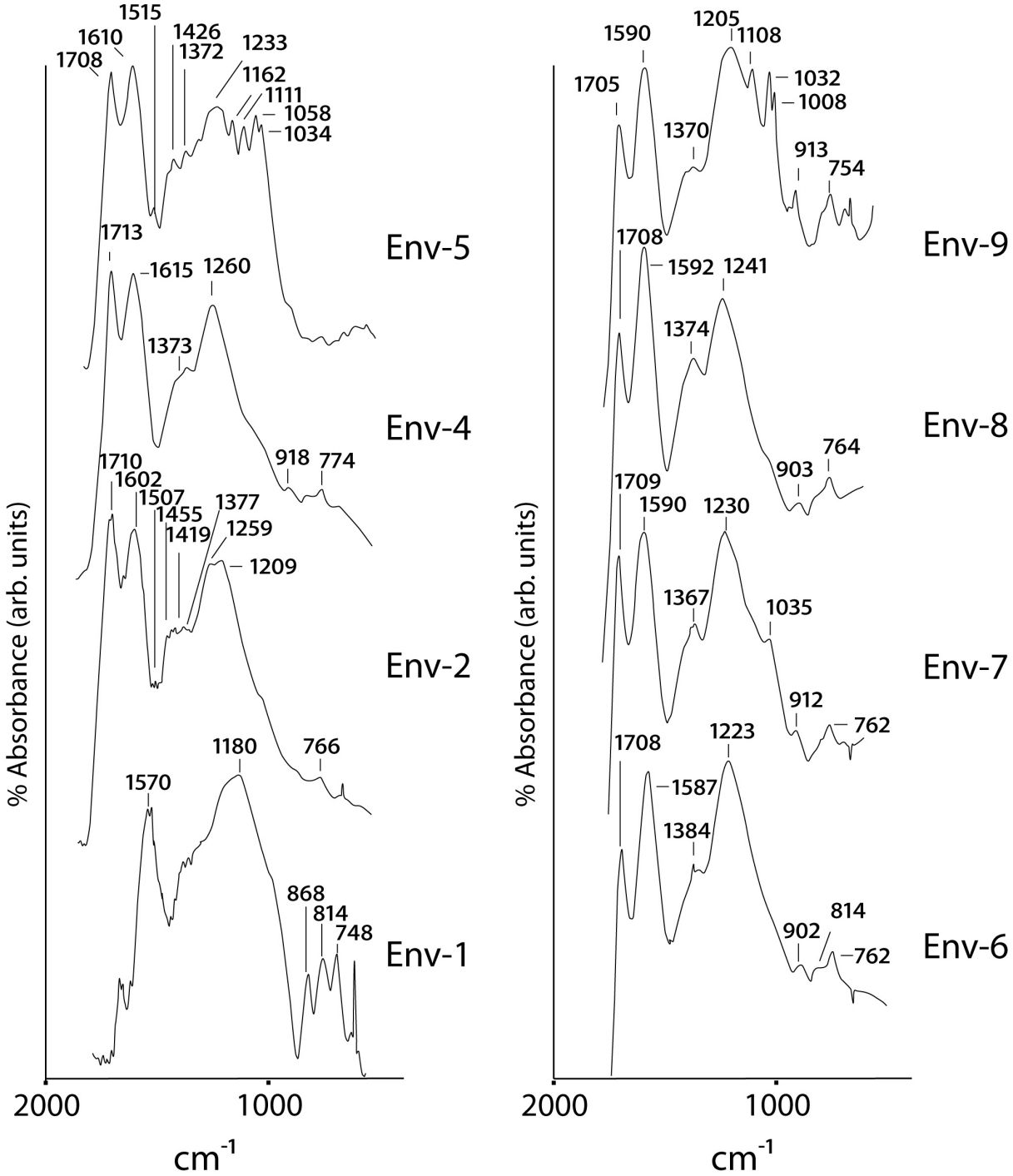


Figure 3: FTIR spectra of Env_{Char} charcoal samples prior to NaOH extraction. Sample labels are given on the figure.

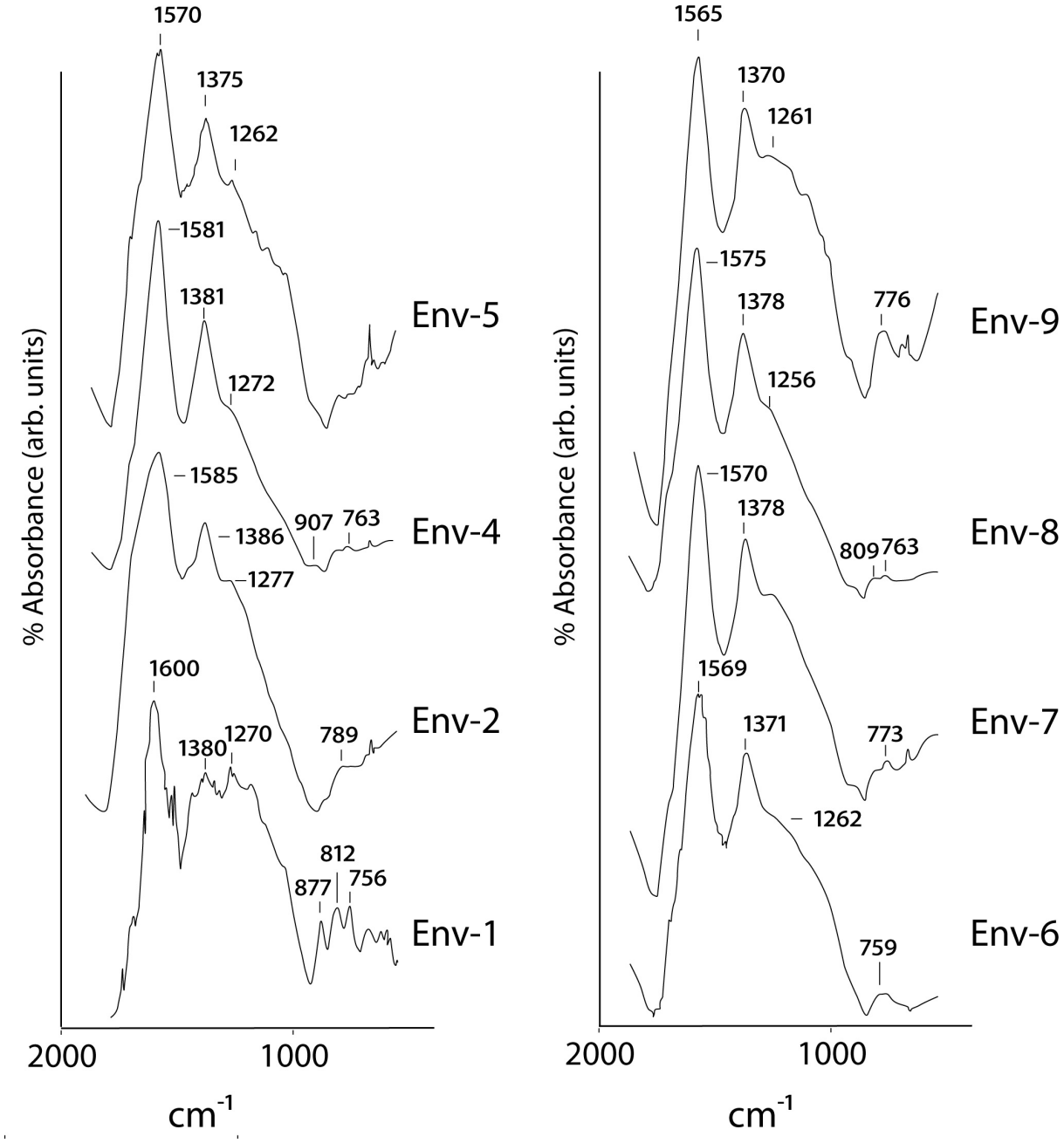


Figure 4: FTIR spectra of Env_{Char} charcoal residue following NaOH extraction. Sample labels are given on the figure.

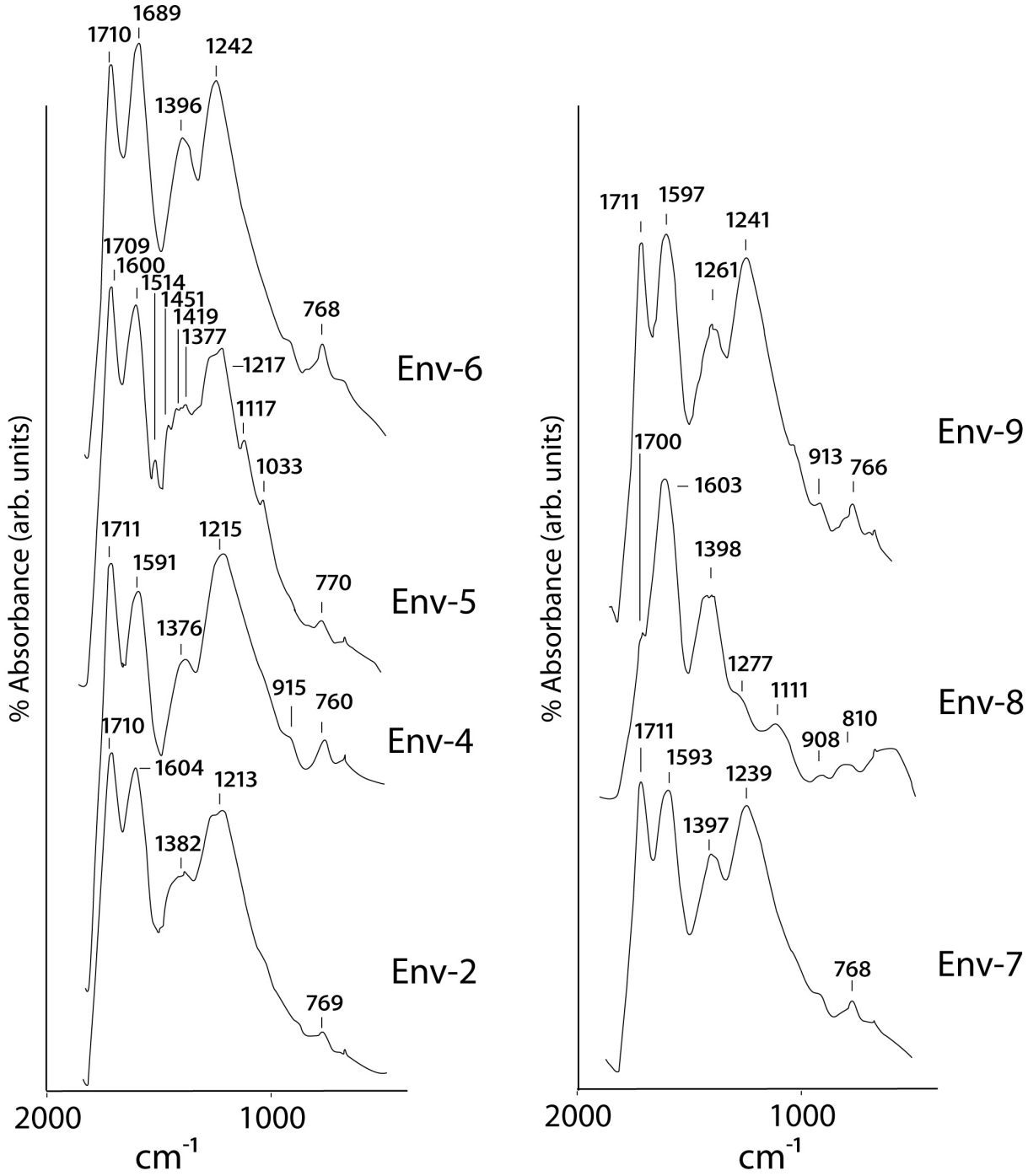
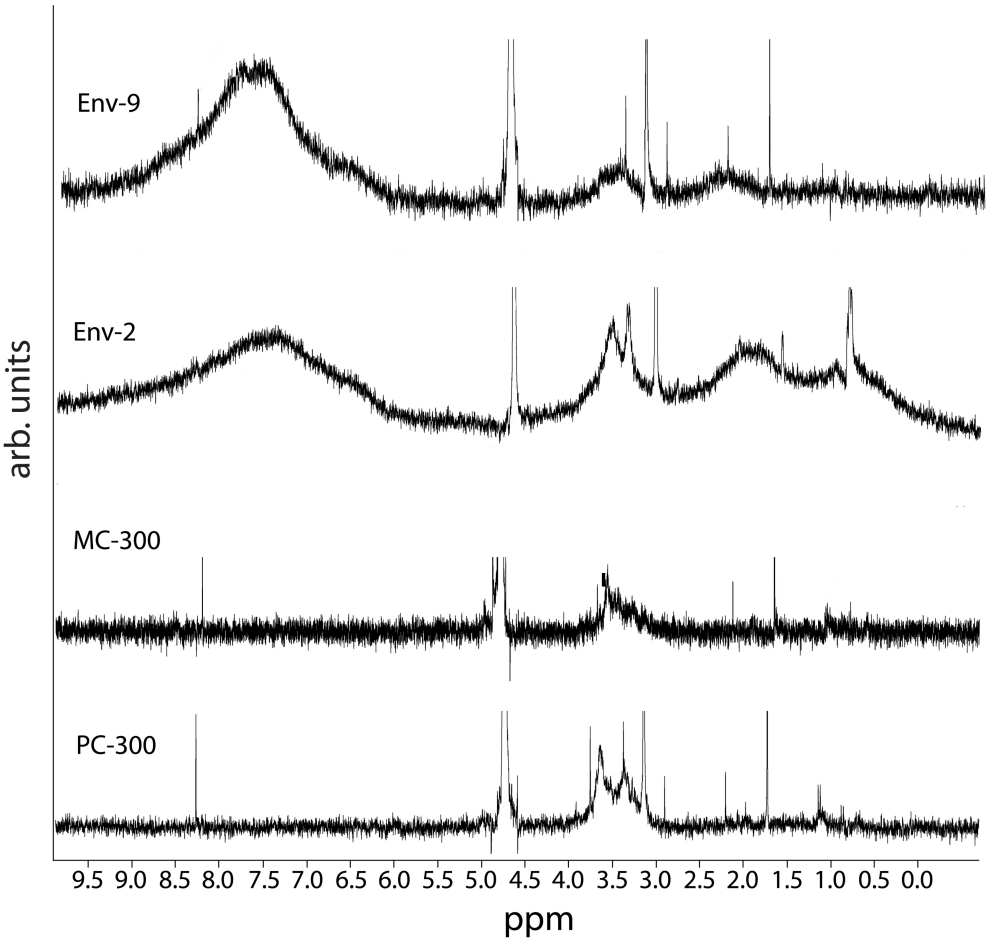


Figure 5: FTIR spectra of HA material extracted from Env_{Char} charcoal samples. Sample labels are given on the figure.



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